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13. ABSTRACT *(Maximum 200 Words)*

This proposal focuses on development of a radically new method for breast magnetic resonance imaging (MRI), which could improve detection of small tumors and reduce the unnecessary biopsies generated by false positives in conventional breast MRI and mammography. This method is based on my group's recent discovery of a significant omission in the decades-old theoretical framework of nuclear magnetic resonance (NMR, the spectroscopic precursor to MRI). We have shown (both theoretically and experimentally) that it is possible to detect strong signals from intermolecular resonances – for example, simultaneously flipping up a water spin at one location while flipping down another water spin 100 μm away–even though such "intermolecular zero-quantum coherences" (iZQCs) would be predicted to be completely impossible to observe in the conventional formulation of NMR or MRI. This fundamentally new physics provides the basis for a potentially extremely useful contrast enhancement technique geared towards early detection and tumor grading. Our previous human work had been restricted to brain imaging; we have now demonstrated iZQC breast imaging on healthy volunteers, with and without fat suppression, and are beginning patient trials.

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Table of Contents

Introduction

Work in my laboratory at the time of grant submission had shown that intermolecular cross-peaks could be generated in vivo, and that these peaks gave enhanced contrast in rat brain images, including tumor enhancement. These cross-peaks arise from dipolar couplings between distant spins in solution, which were previously thought to produce insignificant effects. Instead, we have shown (in five Science papers since 1993, among other places) that they lead to a completely new method for detecting small local variations in the resonance frequency. The overall goal of the research over the entire grant period is to demonstrate that we can enhance signal strength and specificity enough to make this a useful tool for clinical diagnosis of breast tumors. Work in the first grant period focused on transitions to human subjects, signal enhancement, and demonstration of contrast improvement; work in this grant period has focused on transitions to breast imaging, compensation for fat/water frequency difference (absent in brain imaging done to date), and optimization of contrast. Zero-quantum and double-quantum images in healthy subjects have now been demonstrated.

Body

The Statement of Work items relevant for this grant period (from the original proposal), and progress towards the stated goals, is listed below.

Task 1: Characterize intermolecular zero-quantum coherences in samples with susceptibility variations *in vitro*.

c. Develop enhanced imaging sequences (multiple echoes, fat suppression) and test in phantoms and on normal and tumor-containing tissue samples (months 3-36)

Progress on these items:

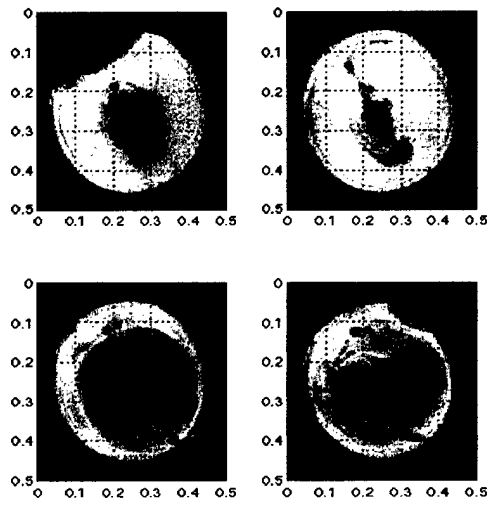


Figure 1. MR microimaging (20x20x250 micron resolution in this case) has been developed and demonstrated on a Varian NMR machine at Princeton. This permits more rapid pulse sequence optimization. The illustration above shows four slices of an earthworm at 10C.

c. Zero-quantum, double-quantum, and multiple echo sequences have been developed with fat suppression and with improved sensitivity. In all cases, the most serious issue with optimizing sequences for breast imaging is compensation for the susceptibility variations between fat and tissue. This was not an issue for brain imaging, but is particularly important in breast, and particularly complex for our sequences.

Sequences are developed for the 4T magnet at the University of Pennsylvania, but we have developed a testing protocol in vitro on a conventional nuclear magnetic resonance spectrometer at Princeton. This spectrometer (a Varian 600 MHz) is easier to program, is capable of giving stronger gradients because of reduced sample size (5 mm tube), and gives excellent sensitivity because of the very high field. This makes pulse sequence optimization much easier. Figure 1 shows a microimaging example (in this case, of an earthworm); this is a very convenient test system because simply cooling the tube (to 10C) is sufficient to keep the sample motionless without harming the specimen.

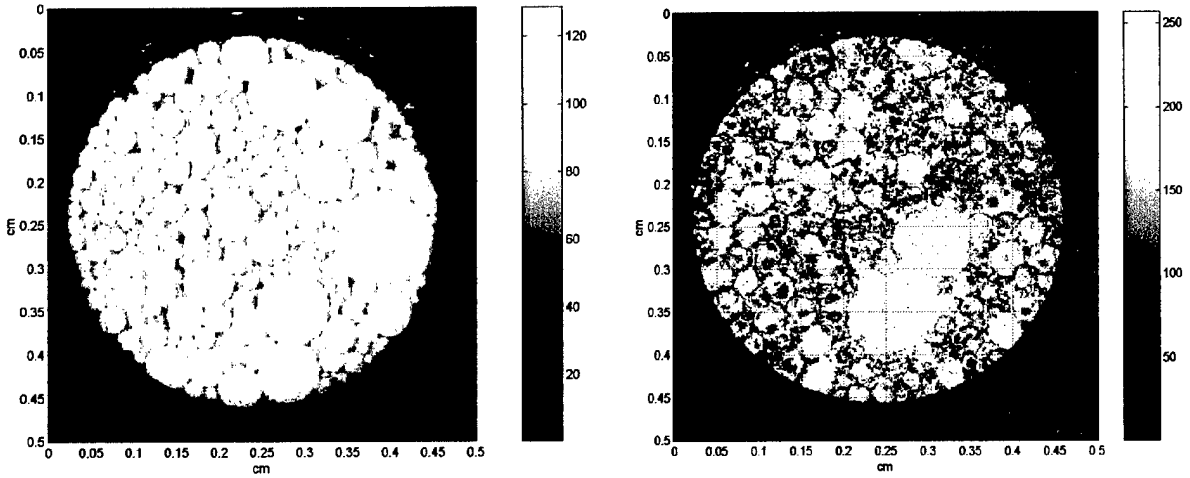


Figure 2 (above) shows conventional (left) and iZQC imaging (right) of model tissue, in this case an oil-water suspension. Note the very large differences in structure for the small droplets: the center of the drop is selective suppressed. This is consistent with our understanding of expected image contrast differences in iZQC images.

Task 2: Demonstrate breast MRI with contrast generated by intermolecular zero-quantum coherences

b. Include iZQC echo-planar studies in ongoing clinical protocols at the University of Pennsylvania on patients with known breast cancer at 4 Tesla (months 3-18)

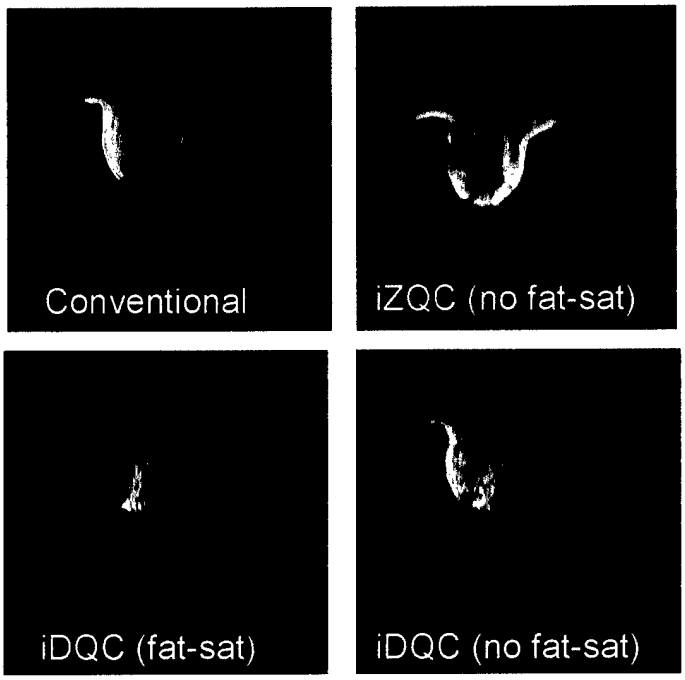
c. Develop data base of iZQC images to compare with conventional MRI, mammograms and biopsy results; evaluate correlations with iZQC signal intensity, linewidth, and nonexponential behavior (months 3-18)

d. Evaluate advanced iZQC imaging sequences at 1.5 Tesla on phantoms (months 12-15)

e. Include iZQC echo-planar studies in ongoing clinical protocols at the University of Pennsylvania on patients with known breast cancer at 1.5 Tesla, and compare with conventional MRI (months 12-24)

Progress: Work is ongoing to optimize pulse sequences for breast coils in use at UPenn. Issues here which are not faced in brain imaging include increased rf inhomogeneity, increased susceptibility variations, and instabilities due to breathing artifacts. All of these effects are automatically compensated in conventional images; the trick is to achieve the same degree of compensation in iZQC/iDQC imaging, which means defeating the automatic routines normally used by the spectrometer.

We have demonstrated iZQC and iDQC breast imaging on normal volunteers, including fat suppression (Figure 3, next page). The contrast differences are consistent with theoretical variations (and with Figure 2 above); for example, notice that boundaries between fat and surrounding tissue are enhanced. The images shown here were acquired in 6 minutes each. These images show motion artifacts which can be reduced by minor breast compression in the coil (far less than in a mammogram). For reasons we do not yet understand, the double-quantum images show more artifacts. Fat suppression is not yet working quite as well as it should; figure 3 uses a conventional fat suppression method (presaturation) which is fine in normal images, but which produced greater signal losses here.



Conventional image: (fast gradient echo, rf-spoiled): TR=150 ms, TE=1.6 ms, 5 mm thick, 256x128 matrix, FOV=24cm

iZQC/iDQC images: TR=3.5 s, TE=50 ms, 10 mm thick, 256x64, FOV=24 cm, t= 8 ms, 2 NEX (G_z-G_y subtraction), Gradient 2 G/cm * 3 ms (D_c = 197 um)

The University of Pennsylvania is processing an appointment for me as Adjunct Professor of Radiology, which will dramatically simply issues associated with instrument access. Over the next several months (until April 2002) we expect to have significantly improved access for clinical demonstrations, in large part because other studies are winding down in preparation for an instrument upgrade. The iZQC sequence is already good enough to use for "highlighting regions with suspect tumors, and we are currently arranging for patients.

Penn has negotiated a complete replacement of their GE research instrument consoles with Siemens instruments. As a result, the 1.5T and 4T instruments we are using will be transformed into Siemens instruments (and the 4T will be lowered to 3T) with full commercial coil sets. This is expected to produce substantial sensitivity and stability improvements.

Task 3: Evaluate intermolecular multiple-quantum coherence contrast as a tool for breast cancer detection

a. As appropriate, test advanced pulse sequences on patients with no prior history of breast cancer and compare to other diagnostic methods (conventional MRI, mammography) (months 12-36).

Progress: task planned for year 3.

Key Research Accomplishments:

–Demonstrated human iZQC and iDQC breast imaging with fast acquision (<5 min for four slices) and enhanced contrast

–Characterized advanced sequences for breast iZQC imaging

Reportable Outcomes:

To date, five conference papers have been submitted or accepted dealing with the research done here. As these papers and subsequent papers are published, copies will be forwarded.

Conclusions:

We have clearly demonstrated that the novel contrast mechanism we proposed for breast MR can be extended to human studies, with acceptable data acquisition times and enhanced contrast. Work is ongoing to prove that these methods actually give better images in breast.

References:

None in this annual report

NewScientist
24 MARCH 2001 WEEKLY £2 US$3.75

An obscure quantum effect promises to revolutionise the way we screen for tumours. Eugenie Samuel **reckons it might one day save her life**

SEEING THE SEEDS OF CANCER

WHEN my grandmother found a lump in her breast at the age of sixty-four, her doctor told her it was nothing to worry about. By the time he realised his mistake, it was too late. "You've killed me," she told him on his final visit before her death.

She had already lost her mother and grandmother to breast cancer. Today, the hereditary nature of some breast cancers is better understood, and cancer researchers are seeking reliable ways to screen women with this genetic predisposition. The available techniques are not perfect: they can still miss a developing cancer, and with it the opportunity for lifesaving intervention. But a recent discovery involving the weird quantum laws that govern the microscopic world of atoms could provide a better solution.

Researchers are now testing a magnetic scanner that creates a strange quantum link between atomic nuclei in different parts of the body. The two linked nuclei act as one, synchronising their movements even if they are centimetres apart. But this strange behaviour only occurs when the tissue under scrutiny does not contain any abnormalities. This means that the quantum link can deliver vital information about any developing health problems. Clinical trials of the technique are already under way, promising to detect tumours earlier than ever before, and to tell malignant and benign tumours apart at a very early stage. Quantum effects used to be an odd abstraction, with little relevance to our daily existence. But one day soon, the weird nature of quantum physics might just save your life.

The potential of this quantum imaging technique first came to light nearly a decade ago when a group of Princeton University researchers, led by chemist Warren Warren, uncovered a flaw in the 50-year-old theory of magnetic resonance imaging (MRI). This technology is currently considered the most sensitive way to screen for breast cancers, and is used as a follow-up when suspiciously dark areas of breast tissue show up on mammograms (see "Screen test", p 42).

Warren and his collaborators were investigating the structure of proteins by hitting small samples of protein molecules in water with complicated sequences of radio pulses. They hoped that the response of the molecules to this radiation would cast light on the structure. But they didn't understand the results they were getting.

The nuclei of certain atoms behave like tiny magnetic spinning tops. In the strong magnetic field of an MRI machine the spinning nuclei all line up with their axes aligned with the magnetic field. When a radio-wave pulse hits the aligned nuclei, it knocks them out of alignment. They carry on spinning, but at an angle—just as a knocked top will continue to spin even when it is leaning over. Having all these spins at an angle to the main field creates a measurable magnetic signal, which fades as the nuclei gradually return to their aligned, equilibrium position.

Warren and his colleagues thought they would be able to learn about the protein's

Illustrations: Chris Draper

structure from the different ways in which this magnetic signal faded when they varied the characteristics of the radio-wave pulse. But the signal in Warren's samples didn't fade in the way that MRI theory suggested it should. "It was even happening in control samples containing pure water," says Warren. "We thought something must be wrong with the instruments."

When the instruments checked out, he thought he might have made a mistake in designing his pulse sequences. So he pared down the experiment until he was using the simplest possible sequence of just two pulses: a radio-wave pulse followed by a short-lived magnetic field pulse called a crusher. The crusher kicks each of the spins by a different amount, and so should leave them in disarray, destroying any measurable magnetic signal in the sample.

But there was a signal. It was only about a tenth as strong as the usual magnetic resonance signals, but it was still more than a thousand times stronger than the background noise. Warren performed the experiment time and again. There was no doubt that the signal was there.

The researchers found the results of these simple sequences so bizarre that they called the sequences "CRAZED". The term has stuck: it is now the accepted name for the pulses that produce these unexpected weak signals. Warren suspects that he wasn't the first researcher to see signals from CRAZED pulse sequences. "When it happened before, people probably thought 'I made a mistake on my sequence'," he says. But Warren was sure of what he was doing and what he was seeing. All he needed now was an explanation.

Having traced the way the unexpected magnetisation gradually faded, Warren used the mathematical technique known as Fourier transform to reveal the different frequency components of the signal. This provided him with the mysterious signal's "fingerprint"—and it looked strangely familiar.

Strange connection

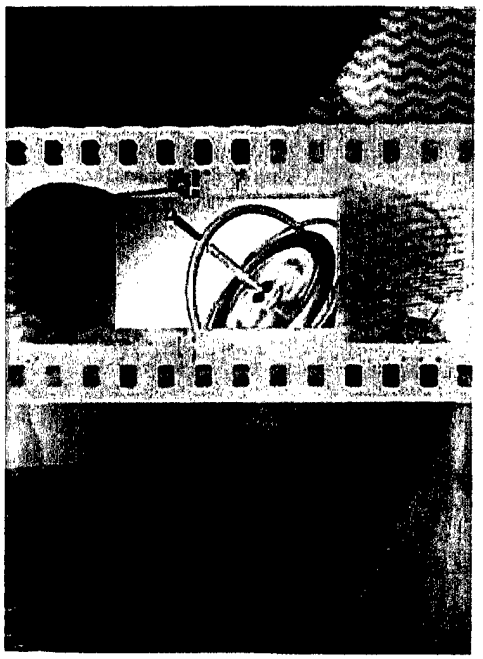
When a pulse of radio waves is produced under certain conditions, its photons acquire a quantum mechanical connection, called "coherence", which keeps them in step with each other. When two coherent photons hit a pair of spinning nuclei they transfer their quantum coherence to the two nuclei. This link binds the nuclei together into one quantum state. One result of this is that the spins will return to equilibrium at exactly the same time, and this simultaneous response gives rise to the characteristic frequency spectrum that Warren recognised.

Warren had only ever seen this link occur between nuclei within the same molecule. But when he examined the quantum coherence created by the CRAZED pulse he was surprised to find that the linked nuclei were micrometres apart, far more than the width of a molecule. Instead of wreaking its normal havoc and destroying all correlation between the spins as it was expected to, the crusher had left the delicate quantum link between two hugely distant spins intact.

Warren eventually worked out what was happening. Although the crusher rotates the axis of each of the spinning nuclei by a different amount, that rotation doesn't destroy every one of the quantum coherences. If it rotated one spin by 10 degrees, say, and another by 370 degrees—a full circle plus 10 degrees—their original relationship would remain the same. Far from knocking all the spins out of alignment, the crusher pulse would allow certain nuclei to remain in step and so maintain their ghostly link.

Since this discovery, Warren and his collaborators have published a string of papers in the journal *Science*, explaining their data and its implications. The most far-reaching of these, Warren has realised, is that it is possible to change the characteristics of the crusher pulse and alter the distance over which the link occurs. The researchers have since established a quantum link between two nuclei that are centimetres apart. "We've even done this between molecules in different test tubes," Warren says.

Although these long-distance correlations are impressive and strange, it is the possibility of going down the scale, and producing quantum coherences between nuclei just one-tenth of a millimetre apart that has got imaging researchers excited. Warren's quantum coherences only form between pairs of nuclei that are in exactly the same environment—which means they must be in tissue that's in the same state of health. So by imaging neighbouring molecules, and finding where the coherences don't form, Warren can pinpoint exactly where the health of the tissue changes. This should allow him to trace out, say, the border of a tumour. The technique will provide a resolution about 50 times finer than the limit of conventional MRI.



SCREEN TEST

THE techniques currently used to screen women for breast cancer are good, but not good enough. X-ray mammography, the basic screening tool, looks for variations in the density of breast tissue. In theory, X-rays can distinguish the higher density of a tumour from that of normal tissue, just as they distinguish bone from muscle. The technique is routinely used to screen women over 50 in Britain. In the US, where missed diagnoses have resulted in lawsuits, mammography screening clinics are not so common.

However, the technique does not work well in younger women, whose breast tissue can have the same density as a developing tumour. This is a problem because many women with a genetic predisposition to breast cancer are young when their tumours begin to form.

Clinicians in Britain and the US are testing the routine screening potential of magnetic resonance imaging (MRI), which is already used to investigate suspicious lumps found through mammography. MRI is superior to mammography because it doesn't rely on density measurements, doesn't give a radiation dose, and it can provide images in "slices" rather than the single view through the breast that you get from X-rays.

But MRI has its own shortcomings: the contrast between healthy and cancerous tissue is slight, so seeing tumours is difficult until they become large and dangerous. Clinicians can inject compounds that increase the contrast, but it doesn't always help. It is still not clear whether MRI screening will improve early cancer detection and provide an alternative to voluntary mastectomies, often used to protect women in high risk groups.

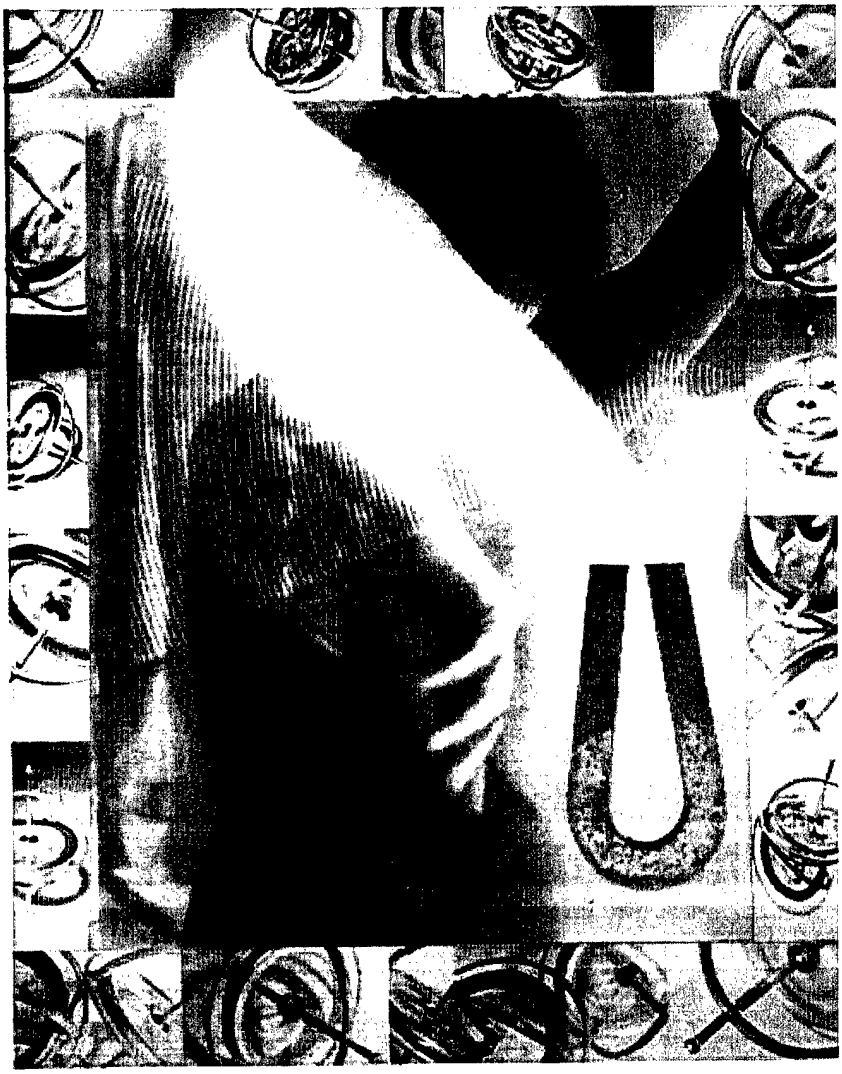
And this is just the start. Because Warren's technique provides an accurate measure of the oxygen concentration in body tissues, it can also diagnose the exact state of a tumour. Tumours use oxygen in a very particular way. The outside of a malignant tumour co-opts its own blood supply from the body to provide a stream of oxygen for growth. But the inside of the tumour has stopped growing and is largely dead and deoxygenated. Most normal tissue has an oxygenation level somewhere between these two extremes.

The oxygenation level of the tissue determines the rate at which its spinning nuclei, disturbed by the radio-wave pulse, will return to their original orientation. Only two spins that return at the same rate will form a quantum coherence and give out the fingerprint signal. At the borders of a tumour, where there is a sharp change in the level of oxygenation, the quantum coherence can't form, and thus there is no signal.

With Mitch Schnall at the Hospital of the University of Pennsylvania, in Philadelphia, Warren has now begun clinical trials of breast tissue imaging using the new quantum states. They have two aims: to pick up breast tumours too small to see with current techniques, and to use the better resolution to pick out more detail in the blood supply. If they can see exactly how the tumour is growing they should be able to determine the level of malignancy without the need to remove a sample of tissue for biopsy—a painful and distressing procedure. The clinical trials will run for a year, and until they are over, Schnall doesn't want to make any concrete claims. "We're not yet in a position to say it's better, but it has the potential for substantial impact," he says.

Researchers at the Institute of Cancer Research at the Royal Marsden NHS Trust hospital in Surrey are also generating images using the new technique. Angelo Bifone and Martin Leach have produced the first quantum coherence images of the human brain and of brain tumours, although they are still learning how to interpret the pictures. "We know there's a lot of new information in there," says Leach, "but we don't know how to use it yet." Nevertheless the team can see attractive possibilities ahead. "If you want to study smaller blood vessels you can just use a pulse that's twice as long or twice as strong—there's nothing like that in conventional MRI," says Bifone.

In his latest study, Bifone has used the new method on the bone of people suspected of having osteoporosis, where the bone becomes porous, or "trabecular". "You can look at holes on different length scales in the trabecular bone to see if and how they're thinning," he says.



'WARREN'S MOTHER DIED OF BREAST CANCER IN THE SAME YEAR HE DISCOVERED THE QUANTUM LINKS'

It's still too soon to tell exactly how good the technique will prove in all these situations, but Warren believes that quantum imaging could eventually save lives. His own mother died of breast cancer the same year he discovered the quantum links. She was diagnosed in 1986, and fought the tumour through surgery and chemotherapy. But nine years later, the cancer recurred and killed her. At the time Warren was some way from his first images. "Today," he says, "she would have been a prime candidate for what we hope we can achieve: aggressive, very early detection."

I can't help wondering whether this quantum imaging would also have saved my grandmother's life. Or whether, one day, the strange nature of quantum physics will come between me and my genetic fate. □

Further Reading: "Multiple spin echoes for the evaluation of trabecular bone quality" by Sylvia Capuani, Angelo Bifone and others, *Magnetic Resonance Materials in Physics, Biology and Medicine* (2001, in press)
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For MRI trial information see: www.icr.ac.uk/cmagres/maribs/maribs.html